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Award Number: W81XWH-05-1-0181

TITLE: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

PRINCIPAL INVESTIGATOR: Georgi Guruli, M.D., Ph.D.
Mark L. Jordan, M.D.

CONTRACTING ORGANIZATION: University of Medicine and Dentistry of New Jersey
Newark, NJ 07107-3001

REPORT DATE: February 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-02-2006	Annual	15 Jan 2005 – 14 Jan 2006

4. TITLE AND SUBTITLE

Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0181

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Georgi Guruli, M.D., Ph.D.
Mark L. Jordan, M.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Medicine and Dentistry of
New Jersey
Newark, NJ 07107-3001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

First annual report for this award. Experiments were conducted as was scheduled in the Statement of Work. So far studies have demonstrated for the first time the presence of endothelin receptors on murine DC, and the fact of endothelin-1 production by murine DC upon stimulation with TNF•. Phenotyping of dendritic cells stimulated with TNF• and treated with endothelin receptor inhibitors demonstrated decreased expression of pro-inflammatory co-stimulatory molecules (CD40, VD80, CD86, CD205, MHC class II) with the blockade of ETA receptors, and no change or mild increase in the expression of co-stimulatory molecules with the blockade of ETB receptors. In vivo administration of endothelin A receptor inhibitor abolished the effect of infectious stimulus to mobilize dendritic cells to draining lymph nodes. Functional studies are under way to further characterize the role of endothelin receptors in the biology of dendritic cells, as well as to study the interaction of dendritic cells and prostate cancer cells, and develop the means of active cell therapy for murine prostate cancer model.

15. SUBJECT TERMS
Prostate cancer, Dendritic cells, Endothelin receptors, Immunotherapy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	22	19b. TELEPHONE NUMBER *(include area code)*

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Introduction:

This study is being conducted for the (i) characterization of the prostate cancer and dendritic cells (DC) interaction; (ii) defining the role of endothelin axis in the maturation of DC, (iii) elucidating the role of endothelin axis in the prostate cancer-DC interaction, and (iv) modification of dendritic cells to be used in the treatment of prostate cancer. Mouse model will be used. This is the report for the first year of the award. Experiments are progressing according to the statement of the work so far.

Body of the Report:

First task was to proceed with the characterization of role of endothelin axis in DC. For this purpose, DC were grown from C57BL/6 mice bone marrow, as was described earlier [1]. Briefly, bone marrow cells were first depleted of RBC with lysing buffer for 2–3 min. The single-cell suspensions then was incubated with a cocktail of Abs (αCD4, αCD8a, and B220) for 1 h at 4°C, followed by incubation with rabbit complement for 30 min at 37°C to deplete cells expressing lymphocyte Ags B220, CD4, and CD8. Cells were then incubated overnight (37°C, 5% CO_2) in six-well plates (Falcon, Franklin Lakes, NJ) at a concentration of 10^6 cells/ml in complete medium, consisting of RPMI 1640, 2 mM L-glutamine, 50 μg/ml gentamicin sulfate, 10 mM HEPES, 10% FBS, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate (Life Technologies). The nonadherent cells were collected by gentle pipetting and resuspended at a concentration of 2.5 x 10^5 cells/ml in complete medium supplemented with 1000 U/ml recombinant murine GM-CSF and recombinant murine IL-4 (R&D system). Cells were cultured in six-well plates (4 ml/well) for 7 days at 37°C in 5% CO_2. Nonadherent DC are collected by gentle pipetting, counted, characterized as described previously [2], and used for further studies.

For the characterization of the general impact of endothelin receptors, dendritic cells were stimulated with TNFα for the endothelin production and the expression of endothelin receptors, since our preliminary data indicated increased expression of endothelin receptors upon stimulation in mice (unpublished data). We have previously demonstrated as well increased production of endothelin-1 (ET-1) by human DC, and increased expression of endothelin receptors [3].

For the characterization of ET-1 production, DC were cultured as described above, and stimulated with TNFα (10ng/ml, added on Day 5)) for 48 hours. After that, supernatant was collected, and ET-1 was measured using ET-1 ELISA kit as described [3]. Stimulated cells produced 1044.2 ± 118.6 pg/ml/mln cells, while nonstimulated cells produced 351.9 ± 131.9 pg/ml/mln cells. The difference was statistically significant (P=0.008).

In preliminary data, we have reported increased expression of the endothelin receptors by murine DC upon stimulation using immunohistochemistry. We were not able to repeat these experiments so far because were not able to obtain endothelin receptor antibodies from Abbott Labs. Recently, antibodies has been found and ordered from Alomone Labs, and these confirmatory experiments will be carried out. We will perform RNA studies as well (gene arrays, and RT-PCR).

Next, changes in phenotype has been evaluated. Murine DC has been stimulated with TNFα on day 5 for 48 hours. Stimulated DC were treated with endothelin receptor

inhibitors BQ-123 (Selective ET_A receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours, and with BQ-788 (Selective ET_B receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours as well. After that, cells were collected, washed, counted and stained for flow cytometry. We have evaluated cells for the expression of CD40, CD80, CD86, MHC class II antigen, and CD205. Results are presented in figures 1-3 (Appendices). Briefly, stimulation with TNFα resulted in the increased expression of these costimulatory molecules (as expected). The blockade of ET_A receptor with BQ-123 induced in general decreased expression of the costimulatory molecules, which was especially significant for CD40 and CD205 (figures 2-3, difference was statistically significant by chi-square test, $P<0.001$). On the other hand, the blockade of ET_B receptor with BQ-788 resulted in no change or increased expression of costimulatory molecules, especially CD40 and CD205 (figures 2-3, difference was statistically significant by chi-square test, $P<0.001$).

One experiment was carried out in the in vivo model: C57BL/6 mice were given ET_A receptor inhibitor, and after 5 days were given foot pad injection with LPS. Mice were sacrificed anesthetic overdose, and lymph nodes were recovered. Nontreated mice, and mice treated with foot injection only served as controls. Collected nodes were crushed. Crushed tissue was resuspended in 20 ml medium, cells collected and passed through the cell strainer. After the lysis of red blood cells, the remaining cells were counted, and labeled with MACS CD11c magnetic beads (Miltenyi Biotech, Auburn, CA). After 15 min of labeling, cell suspension was passed through columns with metallic beads inserted into magnets. Negative cells (washed through) were discarded, columns removed from magnets and CD11c+ cells were collected. Obtained cells were characterized for the expression of costimulatory molecules. Results of this preliminary experiment are presented in figure 4. As it can be seen, LPS induced increased expression of costimulatory molecules, while pretreatment with ET_A receptor inhibitor abolished the effect. Furter experiments are needed to confirm or clarify these results.

One gene array experiment was performed, to assess the influence of prostate cancer cells on DC. Briefly, 7-day-old cultured DC wetre harvested and co-incubated with the murine prostate cancer cell line RM-1 in six-well plates. DC and tumor cells were separated using membrane inserts with 0.4-μm pore size, which exclude direct cell-to-cell contact, but allow free exchange of soluble factors. Specifically, 5×10^5 DC will be placed in six-well plates in 3 ml of medium. One million prostate cancer cells resuspended in 2 ml of medium were placed into the inserts on the top of each well. As controls, DC were coincubated with murine splenocytes. DC were harvested 48 h later, washed, RNA was extracted using RNA extraction minikit, and used for gene arrays. We used mouse 22K Oligo Arrays (Center for Applied Genomics) which is composed of fifteen-thousand 70 mer oligonuclotides corresponding to specific mouse transcripts. The oligonucletides were spotted onto poly-lysine-coated glass microscope slides by using a Gene Machines Omnigrid 100 arrayer (Genomic Solutions, Ann Arbor, Mich.) and SMP3 pins (Telechem, Sunnyvale, Calif.). RNA labeling and hybridization was performed using the 3DNA Array detection Array 350 Kit (Genisphere Inc.) according to the manufacturer's instructions. We used "comparison design" for this experiment, were RNA's were compared to each other directly, without standard (results can be only preliminary). Raw labeled image is presented in figure 5. Preliminary analyze of data demonstrated so far decreased

expression of receptors for IL-12 and interferon gamma in DC incubated with RM-1 cells. More experiments with "reference settings" are scheduled.

Key research Accomplishments:

- Production of ET-1 by murine DC has been documented first time
- The presence of endothelin receptors on murine DC has been shown for the first time
- The influence of endothelin receptor inhibitors on DC phenotype was demonstrated. Other functional experiments (MLR) are on the way, but it seems that ET_A receptors are involved in the activation of DC, driving them towards TH1 response.
- It seems that ET_B receptor stimulation might drive DC toward tolerance, with decreased expression of co-stimulatory molecules. Further studies are needed to clarify the exact role these receptors in DC biology. Functional studies are under way.

Conclusion:

So far experiments have demonstrated the presence of the endothelin receptors on murine DC, which is a novel finding. In addition, the production of ET-1 by murine DC has been demonstrated as well. Experiments suggested the possible role of endothelin receptor inhibitors in the function of DC, which can be useful in the treatment of different diseases, ranging from cancer to transplantation. More in vitro and in vivo experiments are under way to clarify this role.

References:

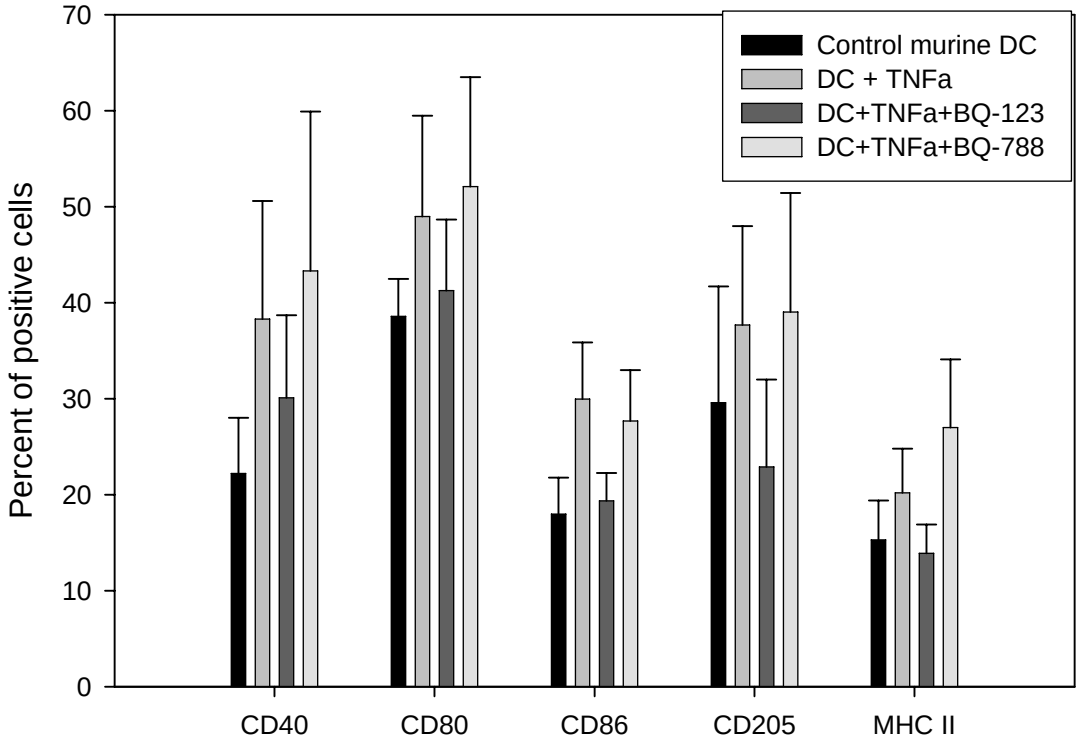
1. Pirtskhalaishvili G, Shurin GV, Gambotto A, Esche C, Wahl M, Yurkovetsky ZR, Robbins PD, Shurin MR. Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. Journal of Immunology. 2000;165:1956-1964

2. Shurin MR, Pandharipande PP, Zorina TD, Haluszczak C, Subbotin VM, Hunter O, Brumfield A, Storkus WJ, Maraskovsky E, Lotze MT. FLT3 ligand induces the generation of functionally active dendritic cells in mice. Cell Immunol. 1997;179:174-184

3. Guruli G, Pflug BR, Pecher S, Makarenkova V, Shurin MR, Nelson JB. Function and survival of dendritic cells depend on endothelin-1 and endothelin receptor autocrine loops. Blood. 2004;104:2107-2115

Appendices:

Figure 1.
Phenotyping of murine dendritic cells (DC) stimulated with TNFα and treated either with ET_A receptor inhibitor (BQ-123) or ET_B receptor inhibitor (BQ-788). Blockade of ETA receptors resulted in the decrease of the costimulatory molecule expression, while ETB receptor blockade was accompanied by mild increase in the expression of costimulatory molecules.



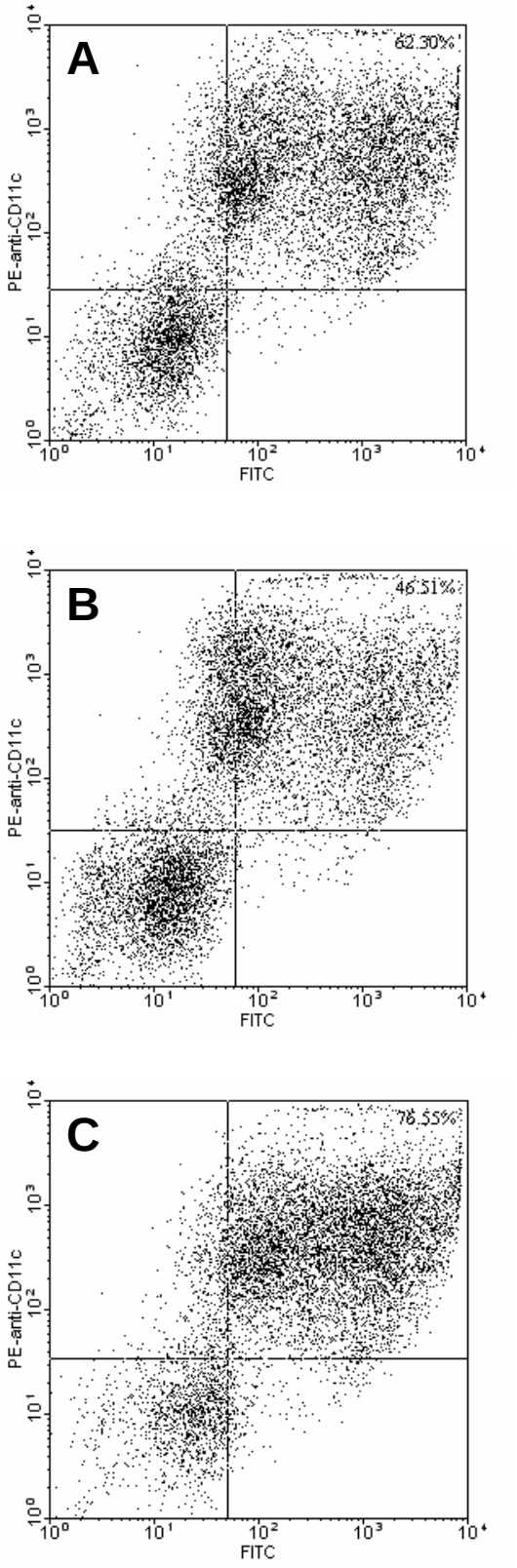


Figure 2.
Expression of CD40 molecules on murine dendritic cells after different treatment (double stained cells – CD11c and CD205).

A. Murine DC stimulated by TNFα.

B. Murine DC stimulated by TNFα and ETA receptor antagonist BQ-123.

C. Murine DC stimulated by TNFα and ETB receptor BQ-788.

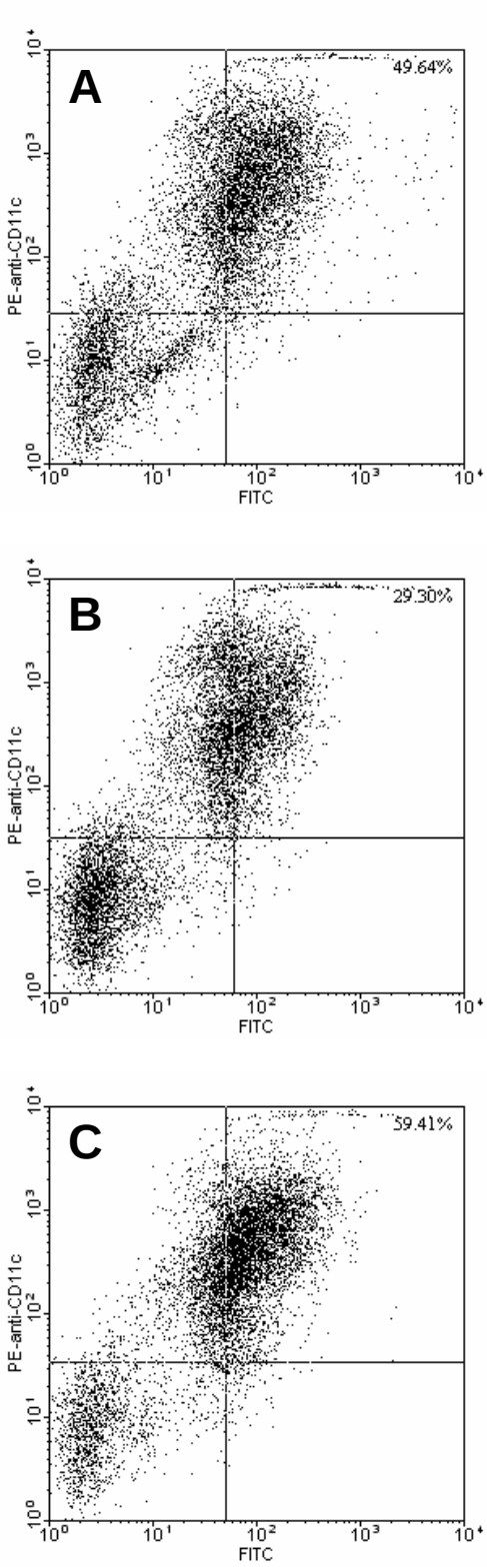


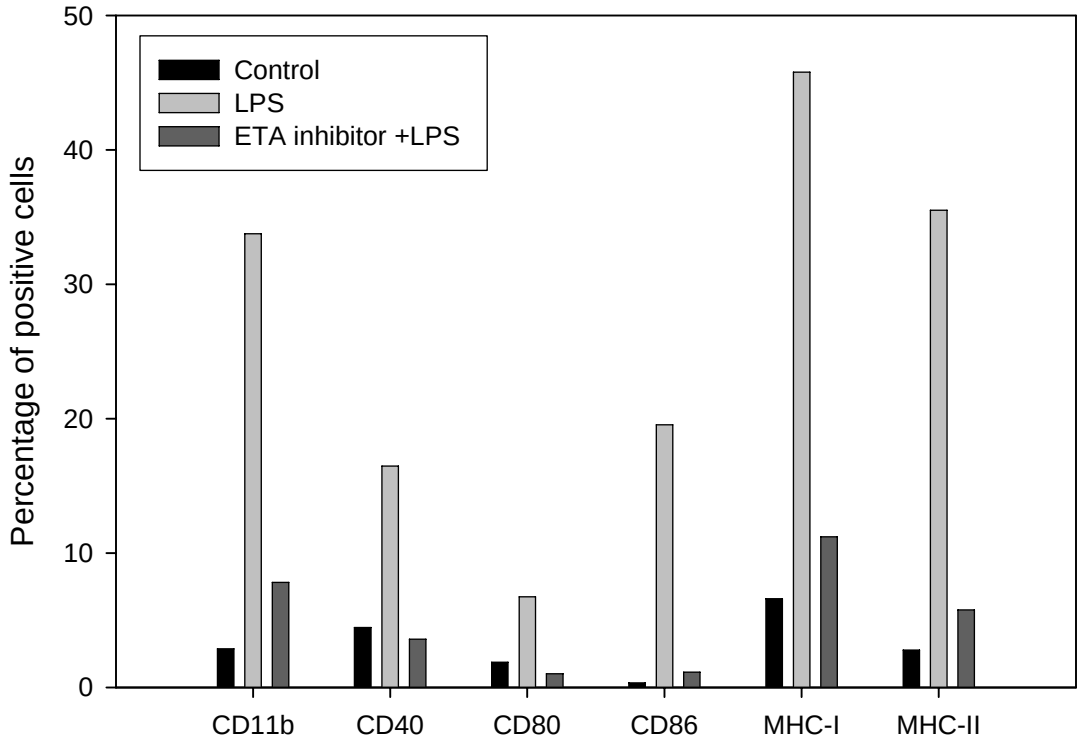
Figure 3.
Expression of CD205 molecules on murine dendritic cells after different treatment (double stained cells – CD11c and CD205).

A. Murine DC stimulated by TNFα.

B. Murine DC stimulated by TNFα and ETA receptor antagonist BQ-123.

C. Murine DC stimulated by TNFα and ETB receptor BQ-788.

Figure 4.
Phenotyping of dendritic cells (CD11c positive) isolated from lymph nodes of the mice (untreated – control, treated with LPS injection, and treated with LPS injection after the pretreatment with ET_A receptor inhibitor). LPS induced increased mobilization of CD11c cells, as was expected. Pretreatment with ET_A inhibitor resulted in decreased mobilization of CD11c positive cells in the lymph nodes.



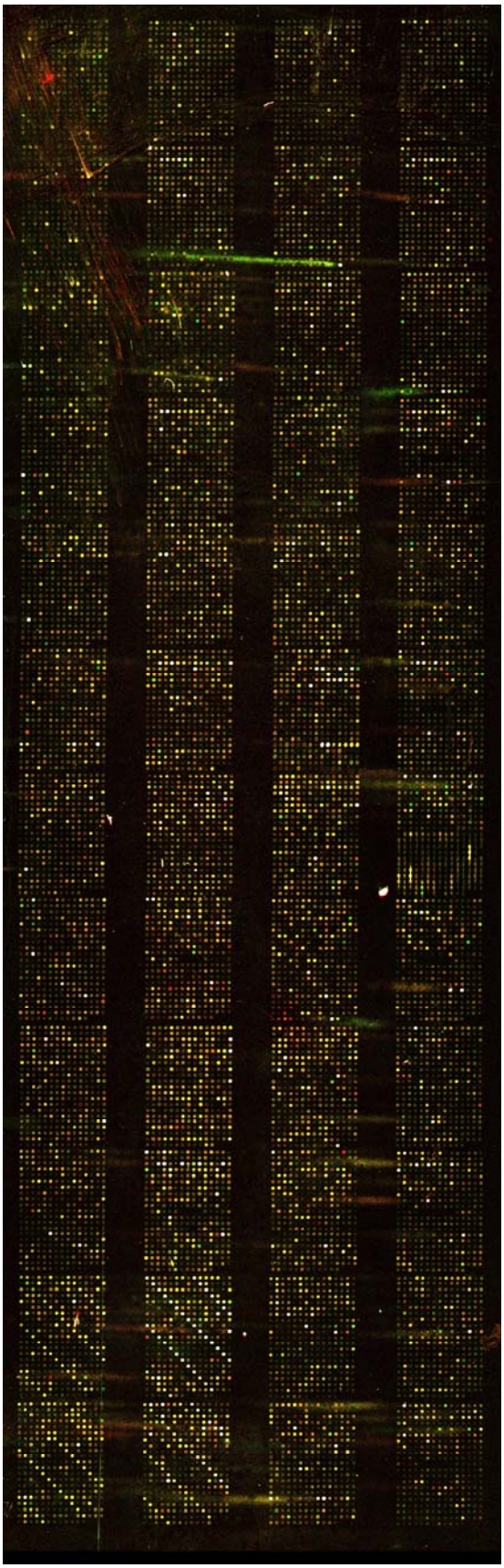


Figure 5.
Spot arrays comparing gene expression in dendritic cells incubated with splenocytes to dendritic cells incubated with murine prostate cancer cells (RM1). Preliminary experiments.

Curriculum Vitae

Name: Georgi Guruli (Pirtskhalaishvili)

Office Address: Division of Urology
Department of Surgery
UMDNJ – New Jersey Medical School
MSB G-528
185 South Orange Avenue
Newark, NJ 07103
Tel: (973) 972-4097
Fax: (973) 972-3892
Email: gurulige@umdnj.edu,

1. Education
   a. Undergraduate N/A
   b. Graduate

      Tbilisi State Medical Institute, Tbilisi, Georgia (former USSR)
      Degree: M.D.
      Date Awarded: June 26, 1983

2. Post Doctoral Training
   a. Internships and Residencies
      i. Clinical ordinatura (residency):
         Location: Georgian Oncological Research Center, Tbilisi, Georgia
         Discipline: Surgical Oncology
         Inclusive Dates: 09/1983 – 09/1985.
      ii. Internship (PGY-I):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Surgery
         Inclusive dates: 07/1995 – 06/1996.
      iii. Residency (PGY-II):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Surgery
         Inclusive dates: 07/1996 – 06/1997.
      iv. Residency (PGY-III – PGY-VI):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Urology
         Inclusive dates: 07/1997 – 06/2001.

b. Research Fellowships

i. Research Fellow:
National Oncological Research Center, Moscow, USSR
Discipline: Urologic Oncology
Inclusive Dates: 09/1987 – 12/1990.
Ph.D. Degree awarded on 12/08/1990.

ii. Research Fellow:
University of Pittsburgh School of Medicine, Pittsburgh, PA
Discipline: Urologic Oncology
Inclusive Dates: 07/2001 – 11/2003.

3. Licensure (state, specialty, issue date, expiration date)

a. Commonwealth of Pennsylvania – Medical Physician and Surgeon, MD 067593 L, Initial License Date: 03/05/1999. Expiration date: 12/31/2004.

b. State of New Jersey – Medical Doctor, License # 25MA07655400, Initial license date: 09/08/2003. Expiration date: 06/30/2007

4. University Appointments:

Department: Urology
University of Pittsburgh School of Medicine
Title: Research Fellow
Inclusive dates: 07/2001 – 11/2003.

Department: Surgery, Division of Urology
UMDNJ – New Jersey Medical School
Title: Assistant Professor
Inclusive dates: 12/2003 – present.

5. Hospital Appointments

Department of Surgery, Division of Urology
Hospital Name: Georgian Oncological Research Center
Title: Ward physician
Inclusive dates: 1985-1995

Department of Surgery, Division of Urology
University Hospital, Newark
Title: Attending physician
Inclusive dates: 12/2003 – present.

6. Awards and Honors

1977 Gold Medal (Highest Honors), High School #1, Tbilisi, Georgia

1983	Highest Honors ("Red Diploma"), Tbilisi State Medical Institute, Georgia
1998	Second Prize, Clinical Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania
1999	Pfizer Scholars in Urology Award.
1999	Best Basic Science Paper Award, 51st Annual Meeting of Northeastern Section, AUA. Bermuda, UK.
1999	First Prize, Basic Research Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania.
2000	Resident Prize Essay Award, 52nd Annual Meeting of Northeastern Section, AUA. Pittsburgh, USA.
2002	Sylvia Sorkin Greenfield Award, for the best paper published in *Medical Physics.*
2004	AUA travel Award to attend NIDDK Clinical Research Meeting

7. Principal Clinical and Hospital Service Responsibilities:

Hospital Name: Georgian Oncological Research Center, Tbilisi, Georgia
Department or Service: Urology
Responsibilities – Admission of patients in the hospital, preoperative evaluation and designing of treatment plan, administration of treatment (surgical or medical), postoperative care in the hospital.
Inclusive Dates: 1985 – 1995.

Hospital Name: University Hospital, Newark, NJ
Department or Service: Surgery (Urology)
Responsibilities – Admission of patients in the hospital, evaluation and elaboration of treatment plan, administration of treatment, post-treatment follow-up.
Inclusive Dates: 12/2003 – present.

8. Ad Hoc Reviewer:
International Journal of Cancer
American Cancer Society
Medical Science Monitor
Grant reviewer for NIH

9. Memberships, Offices and Committee Assignments in professional Societies

i. European Association of Urology
Active Member
1996 – 2000.

ii. American Urological Association
Candidate Member
Dates: 1997 – 2001.
Associate Member
Dates: 2002 – Present.

iii. American Association for Cancer Research
Associate Member
Dates: 1999 – 2004.
Active Member – since 2005.

10. Major Research Interests: Brief Narrative Description in full sentences

b. Prostate cancer:
Relationship and interaction between prostate cancer and dendritic cells (DC), the major antigen-presenting cells. To study the mechanisms of prostate cancer-induced DC suppression, and design the ways of protecting DC from apoptosis. Development of DC-based therapies of advanced prostate cancer.

c. Immunomodulation and the role of endothelin-1 (ET-1) and its receptors in the generation of immune response, in particular, the role of endothelin axis in affecting of DC function.

11. Grant History (No proposed or pending funding, only full awards)

a. Principal Investigator

i. Funding Organization: American Foundation for Urologic Disease / American Urological Association Research Scholar Program
Title of Award: The Endothelin Axis: Signaling Pathways and Maximizing Efficacy in the Treatment of Advanced Prostate Cancer.,
Inclusive dates of Funding: 07/2001 – 06/2003.
Direct costs awarded: $44,000.
Total amount awarded: $44,000.

ii. Funding Organization: Department of Defence, Physician Research Training Grant
Title of Award: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment.,
Inclusive dates of Funding: 2005 – 2009.
Direct Costs awarded: $449,668
Total amount awarded: $699,232.

b. Co-Investigator

i. Funding Organization: University of Pittsburgh Prostate and Urologic
Cancer Center Pilot Project (Co-PI)
Title of Award: Effective Protection of Human Dendritic Cells from Prostate Cancer Induced Cell Death.
Inclusive dates of Funding: 1999-2000.
Total amount awarded: $10,000.

ii. Funding Organization: The Pittsburgh Foundation Program for Medical Research
Title of Award: New Approach for Prostate Cancer Therapy: Dendritic Cells Protected from Tumor-Induced Death.
Inclusive dates of Funding: 1999-2002.
Total amount awarded: $148,132.

iii. Funding Organization: Department of Defense (DAMD17-00-1-0099 P1832735).
Title of Award: Immune Gene Therapeutic Correction and Protection of Disordered Dendritic Cells in Prostate Cancer.
Inclusive dates of Funding: 1999-2002.
Total amount awarded: $471,339.

12. Articles

1. Gotsadze, D. T. & **Pirtskhalaishvili, G. G**. (1988). [Diagnosis and treatment of regional metastasis of penile cancer] [Russian]. *Urologiia i Nefrologiia*, 48-51.

2. Gotsadze, D. T., Daneliia, E. V. & **Pirtskhalaishvili, G. G.** (1988). [Lymphogenic metastasis in penile cancer] [Russian]. *Voprosy Onkologii* **34**, 1501-1504.

3. Gotsadze, D. T., Nemsadze, G. G., Chigogidze, T. G., Daneliia, E. V., **Pirtsakhalaishvili, G. G.** & Chovelidze Sh, G. (1990). [A method for forming a large-intestine reservoir for the urine] [Russian]. *Urologiia i Nefrologiia*, 35-39.

4. Gotsadze, D., Mosidze, B., Chigogidze, T., Nemsadze, G., Chovelidze, S. & **Pirtskhalaishvili, G.** (1990). [Surgical aspects for the construction of colonic urinary reservoirs] [Georgian]. *Sakartvelos Sameditsino Moambe*, 36-41.

5. Matveev, B. P., Shipilov, V. I., Gotsadze, D. T., Abdushelishvili, K. O. & **Pirtskhalaishvili, G. G**. (1990). [The incidence of bladder tumor recurrences after transurethral resection during combined treatment] [Russian]. *Urologiia i Nefrologiia*, 53-56.

6. Shipilov, V. I. & **Pirtskhalaishvili, G. G**. (1990). [Transurethral resection in the treatment of locally advanced cancer of the bladder] [Russian]. *Voprosy Onkologii* **36**, 1369-1371.

7. Gotsadze, B. T., Nemsadze, G. G., Mosidze, B. A., **Pirtskhalashvili, G. G**., Chovelidze Sh, G. & Daneliia, E. V. (1991). [A "dry" abdominal urinostoma] [Russian]. *Vestnik Khirurgii Imeni i - i - Grekova* **146**, 120-122.

8. Matveev, B. P., Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1991). [The results of cystectomy in bladder cancer] [Russian]. *Voprosy Onkologii* **37**, 1095-1098.

9. Gotsadze, D. T., Daneliia, E. V., **Pirtskhalaishvili, G. G.** & Arutiunov, E. T. (1991). [Malignant tumors of the testis in the Georgian SSR] [Russian]. *Voprosy Onkologii* **37**, 25-28.

10. Gotsadze, D., **Pirtskhalaishvili, G.,** Danelia, E., Chovelidze, S., Zangaladze, L. & Zedginidze, T. (1991). [Abdominal reservoir as an alternative to cutaneous urinary diversion] [Russian]. *Diagnosis and treatment of genitourinary tumors*. B. P. Matveev (Ed.). Moscow**:** 54-59.

11. Daneliia, E. V., Gotsadze, D. T. & **Pirtskhalaishvili, G. G**. (1992). [The lack of knowledgeability of men about testicular tumors as a cause for the late diagnosis of this disease] [Russian]. *Voprosy Onkologii* **38**, 1254-1258.

12. Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1992). [The quality of life of patients after cystectomy for cancer] [Russian]. *Voprosy Onkologii* **38**, 489-493.

13. Matveev, B. P., Gotsadze, D. T. & **Pirtskhalaishvili, G. G.** (1993). [The causes of mortality following cystectomy for bladder tumor] [Russian]. *Urologiia i Nefrologiia*, 20-22.

14. Gotsadze, D. T., **Pirtskhalaishvili, G. G**., Chovelidze Sh, G. & Chigogidze, T. G. (1993). [The results of the diversion of urine into a large-intestine reservoir] [Russian]. *Urologiia i Nefrologiia*, 28-30.

15. Gotsadze, D., Charkviani, L., Nemsadze, G., Tsintsadze, I. & **Pirtskhalaishvili, G.** (1994). Continent urinary diversion (Gotsadze Pouch) after pelvic exenteration for gynaecological malignancies. *European Journal of Gynaecological Oncology* **15**, 369-371.

16. Gotsadze, D. T., **Pirtskhalaishvili, G. G**. & Alkhanishvili, K. B. (1995). [A detubularized reservoir for the urine made from the small intestine] [Russian]. Urologiia i Nefrologiia, 38-41.

17. Gotsadze, D. & **Pirtskhalaishvili, G**. (1995). Abdominal reservoirs for continent urinary diversion. *Journal of Urology* **154**, 985-988.

18. Gotsadze, D. T., **Pirtskhalaishvili, G. G**. & Alkhanishvili, K. B. (1996). [The choice of urinary diversion in tumors of the small pelvis] [Russian]. *Voprosy Onkologii* **42**, 82-84.

19. Gotsadze, D. & **Pirtskhalaishvili, G.** (1998). Meckel's diverticulum as a continence mechanism. *Journal of Urology* **160**, 831-832.

20. **Pirtskhalaishvili, G.,** Konety, B. R. & Getzenberg, R. H. (1999). Update on urine-based markers for bladder cancer. How sensitive and specific are the new noninvasive tests? *Postgraduate Medicine* **106**, 85-86, 91-94.

21. **Pirtskhalaishvili, G.**, Getzenberg, R. H. & Konety, B. R. (1999). Use of urine-based markers for detection and monitoring of bladder cancer. *Techniques in Urology* **5**, 179-184.

22. **Pirtskhalaishvili, G**. & Shurin, M. R. (2000). Dendritic cells in the treatment of prostate cancer. *Cancer Research Alert* **1**, 89-91.

23. **Pirtskhalaishvili, G**., Shurin, G. V., Esche, C., Cai, Q., Salup, R. R., Bykovskaya, S., Lotze, M. T. & Shurin, M. R. (2000). Cytokine-mediated protection of human dendritic cells from prostate cancer-induced apoptosis is regulated by the Bcl-2 family of proteins. *British Journal of Cancer* **83**, 506-513.

24. **Pirtskhalaishvili, G**. & Nelson, J. B. (2000). Endothelium-derived factors as paracrine mediators of prostate cancer progression. *Prostate* **44**, 77-87.

25. **Pirtskhalaishvili, G**., Shurin, G. V., Gambotto, A., Esche, C., Wahl, M., Yurkovetsky, Z. R., Robbins, P. D. & Shurin, M. R. (2000). Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. *Journal of Immunology* **165**, 1956-1964.

26. Esche, C., Shurin, G. V., Kirkwood, J. M., Wang, G.-Q., Rabinowich, H., **Pirtskalaishvili, G**. & Shurin, M. R. (2001). TNF-a-promoted expression of Bcl-2 and inhibition of mitochondrial cytochrome C release mediate resistance of mature dendritic cells to melanoma-induced apoptosis. *Clinical Cancer Research* **7**, 974s-979s.

27. **Pirtskhalaishvili, G.,** Shurin, G. V., Esche, C., Trump, D. L. & Shurin, M. R. (2001). TNF- protects dendritic cells from prostate cancer-induced apoptosis. *Prostate cancer and prostatic diseases* **4**, 221-227.

28. **Pirtskhalaishvili, G**., Hrebinko, R. L. & Nelson, J. B. (2001). The treatment of prostate cancer: an overview of current options. *Cancer Practice* **9**, 295-306.

29. Pan, Y., Lavelle, J. P., Bastacky, S. I., Meyers, S., **Pirtskhalaishvili, G**., Zeidel, M. L. & Farkas, D. L. (2001). Detection of tumorigenesis in rat bladders with optical coherence tomography. *Medical Physics* **28**, 2432-2440.

30. Shurin, G. V., Aalamian, M., **Pirtskhalaishvili, G.**, Bykovskaia, S., Huland, E., Huland, H. & Shurin, M. R. (2001). Human prostate cancer blocks the generation of dendritic cells from CD34+ hematopoietic progenitors. *European Urology* **39 Suppl 4**, 37-40.

31. **Pirtskhalaishvili, G**. & Nelson, J. B. (2002). The Endothelin Receptor: A Novel Target for Anticancer Therapy. *American Journal of Cancer* **1**, 81-91.

32. Konety B.R., **Pirtskhalaishvili G.** (2002) Transitional cell carcinoma, renal. In: Cunha BA, Geibel J, Leslie SW, Marriott HJL, Schulman P, Shulman LP, Soreff S, Zevitz ME, eds. *Medicine, OB/Gyn, Psychiatry, and Surgery - An online medical reference.* Vol. 3: Emedicine Journal; http://www.emedicine.com/med/topic2003.htm.

33. Makarenkova V.P., Shurin G.V., Tourkova I.L., Balkir L., **Pirtskhalaishvili G.**, Perez L., Gerein V., Siegfried J.M., Shurin M.R. (2003) Lung cancer-derived bombesin-like peptides down-regulate the generation and function of human dendritic cells. *Journal of Neuroimmunol*ogy **145**:55-67

34. **Guruli G**, Pflug BR, Pecher S, Makarenkova V, Shurin MR, Nelson JB. (2004) Function and survival of dendritic cells depend on endothelin-1 and endothelin receptor autocrine loops. *Blood* **104**: 2107-15.

35. Joel B. Nelson, Michael S. Udan, **Georgi Guruli** and Beth R. Pflug: Endothelin-1 Inhibits Apoptosis in Prostate Cancer; *Neoplasia* **7:** 631-637.

13. Books, Monographs and Chapters

1. Gotsadze D, Mosidze B, Nemsadze G, Chovelidze S, **Pirtskhalaishvili G.** [Construction of colonic reservoir for urinary diversion] [Russian]. *Methodical Recommendations*. Tbilisi; 1990.

2. **Pirtskhalaishvili G,** Nelson JB. Endothelins. In: Creighton T, ed. *Encyclopedia of Molecular Medicine*. New York: John Wiley & Sons; 2002.

3. Zeidel ML, **Pirtskhalaishvili G**. Urinary tract obstruction. In: Brenner MB, ed. *The Kidney*. Vol. 2 (ed 7th). Philadelphia: Saunders; 2004:1867-1893

14. Abstracts

1. Gotsadze, D., **Pirtskhalaishvili, G**., Chovelidze, S. & Nemsadze, G. (1992). Indications for construction of artificial valve for colonic reseroirs. In *Proceedings of Continent Urinary Recinstruction*. First International Meeting, Lund, Sweden. 10-12 June 1992 (p. 149). Scandinavian Journal of Urology and Nephrology, Suppl.

2. Gotsadze, D. T., Nemsadze, G. G., Gvamichava, R. R. & **Pirtskhalaishvili, G**. (1994). Dry umbilical urinostoma after pelvic exenterative surgery. In *2nd International conference on Colo-Rectal Tumours*, Milan, Italy. 11-14 September 1994 (p. F3).

3. Gotsadze, D., Chovelidze, S. & **Pirtskhalaishvili, G**. (1994). Combined cytoreductive organ-spared surgical treatment in patients requiring cystectomy. In *All-Russian Conference on Oncourology*, Obninsk, Russia. 6-7 October 1994 (pp. 63-64).

4. Gotsadze, D., Charkviani, L., Nemsadze, G., Tsintsadze, I., **Pirtskhalaishvili, G**. & Alkhanishvili, K. (1995). Ileal urinary reservoir (Gotsadze Pouch) after exenteration for gynaecological malignancies. In *9th International Meeting of Gynaecological Oncology (ESGO)*, Knokke, Belgium. 9-12 May 1995 (p. 63). European Society of Gynaecological Oncology.

5. **Pirtskhalaishvili, G.** & Hrebinko, R. L. (1998). The prognostic utility of prostate specific antigen density (PSAD). In *50th Anniversary Meeting of the Notheastern Section, American Urological Association*, Toronto, Canada. 18-21 October 1998 (p. 132). Northeastern section, American Urological Association.

6. Hrebinko, R. L. & **Pirtskhalaishvili, G**. (1998). Reliability of ultrasound-determind prostate volumes. In *50th Anniversary Meeting of the Notheastern Section, American Urological Association*, Toronto, Canada. 18-21 October 1998 (p. 140). Northeastern section, American Urological Association.

7. **Pirtskhalaishvili, G.**, Hrebinko, R. L., Robbins, P., Gambotto, A., Lotze, M. T. & Shurin, M. R. (1999). Intratumoral injection of dendritic cells protected from tumor-induced apoptosis causes inhibition of prostate cancer growth in mice. In *Joint Annual Meeting of the New England and Northeastern Sections of the American Urological Association*, Southampton, Bermuda. 31 October - 4 November 1999 (p. 200). Northeastern section, American Urological Association.

8. Konety, B., **Pirtskalashvili, G**., Hershberger, P., Johnson, C. & Getzenberg, R. (1999). Vitamin D induced apoptosis of bladder tumor cells in vitro. In *AUA 94th Annual Meeting*, Dallas, Texas. 1-6 May 1999 (p. 119). American Urological Association.

9. **Pirtskhalaishvili, G**., Salup, R. R., Esche, C., Lotze, M. & Shurin, M. R. (1999). Protection of Human dendritic cells from prostate cancer-induced apoptosis by IL-12 and IL-15. In *94th Annual Meeting of the American Urological Association*, Dallas, Texas. 1-6 May 1999 (p. 126). American Urological Association.

10. **Pirtskhalaishvili, G**., Salup, R. R., Esche, C., Lotze, M. T. & Shurin, M. R. (1999). Induction of apoptosis in human dendritic cells by prostate cancer and the mechanisms of protection. In *90th Annual Meeting of the American Association for Cancer Research*, Philadelphia, Pennsylvania. 10-14 April 1999 (p. 322). American Association for Cancer Research.

11. Salup, R. R., **Pirtskhalaishvili, G**., Deng, D. H., Batthacharya, R., Tran, S. & Lotze, M. T. (1999). Vaccination with plasmid DNA encoding for a truncated HER2/neu protein prevents the growth of prostate cancer in rats and induces a tumor antigen specific immune response in vivo. In *90th Annual Meeting of the American Association for Cancer Research*, Philadelphia. 10-14 April 1999 (p. 256). American Association for Cancer Research.

12. Konety, B. R., Lavelle, J. P., **Pirtskhalaishvili, G**., Calleary, J. G., Meyers, S. A., Ramage, R., Dhir, R., Zeidel, M. L. & Getzenberg, R. H. (2000). Evaluation of vitamin D in the prevention and treatment of bladder cancer. In *95th Annual Meeting of the American Urological Association*, Atlanta, Georgia. 29 April - 4 May 2000 (p. 120). American Urological Association.

13. **Pirtskhalaishvili, G**., Gambotto, A., Esche, C., Yurkovetsky, Z. R., Lotze, M. R. & M.R., S. (2000). IL-12 and Bcl-x_L gene transfection of murine dendritic cells protects them from prostate-cancer induced apoptosis and improves their antitumor activity. In *95th Annual Meeting of the American Urological Association*, Atlanta, Georgia. 29 April - 4 May 2000 (p. 105). American Urological Association.

14. **Pirtskhalaishvili, G**., Gambotto, A., Yamabe, K., Lotze, M. T. & Shurin, M. R. (2000). Protection of dendritic cells (DC) from tumor-induced apoptosis increases the the efficacy of DC-based therapy in a murine prostate cancer model. In *91st Annual Meeting of the American Association for Cancer Research*, San-Francisco, California. 1-5 April 2000 (p. 43). American Associaon for Cancer Research.

15**. Pirtskhalaishvili, G**., Lavelle, J. P., Calleary, J. G., Meyers, S. A., Ramage, R., Bastacky, S. I. & Zeidel, M. L. (2000). Physiological properties of the urothelium during methyl-nitroso-urea (MNU) induced carcinogenesis. In *52nd Annual Meeting, The Northeastern Section of the AUA*, Pittsburgh, Pennsylvania. 14-17 September 2000 (p.140). Northeastern Section, American Urological Association.

16. Bhattacharaya, R., Prawoko, I., Morgan, M., **Pirtskhalaishvili, G**., Soto, J. & Salup, R. R. (2001). Vaccination with plasmid DNA encoding for HER2/neu-EGFP fusion protein immunizes rats against prostate cancer. In *92nd Annual Meeting of the American Association for Cancer Research*, New Orleans, Louisiana. 24-28 March 2001 (p. 819). American Association for Cancer Research.

17. Aalamian, M., **Pirtskhalaishvili, G**., Yamabe, K., Esche, C., Shurin, G. V., Huland, E., Huland, H. & Shurin, M. R. (2002). Prostate cancer suppresses dendropoiesis both in vitro and in vivo. In *AUA 97th Annual Meeting*, Orlando, Florida. 25-30 May 2002 (p. 136). American Urological Association.

18. **Pirtskhalaishvili, G**., M.athis, C., Arlotti, J. A., Pflug, B. R. & Nelson, J. B. (2002). PET imaging of prostate cancer through manipulation of fatty acid synthase. In *AUA 97th Annual Meeting*, Orlando, Florida. 25-30 May 2002 (p. 362). American Urological Association.

19. Shurin, M. R., **Pirtskhalaishvili, G**., Tourkova, I. L., Yurkovetsky, Z. R., Perez, L., Makarenkova, V. P., Balkir, L. & Shurin, G. V. (2002). The dendritic cell system under tumor surveillance. In *The 2nd International Conference on Tumor Microenvironment: Progression, Therapy and Prevention*, Baden, Austria. 25-29 June 2002 (p.94).

20. **Pirtskhalaishvili, G**., Pflug, B. R., Pecher, S., Makarenkova, V., Shurin, M. R. & Nelson, J. B. (2003). Presence of an endothelin-1/ETA autocrine loop in the maturation, differentiation and survival of human dendritic cells. In *94th Annual Meeting of the American Association for Cancer Research*, Washington, DC. 11-14 July 2003 (p. 555). American Association for Cancer Research.

21. **Guruli, G.**, Pflug, B.R., Shurin, M. R. and Nelson, J. B.: Gene expression changes in dendritic cells after their interaction with prostate cancer cells. In *96th Annual Meeting of the American Association for Cancer Research*, Anaheim, CA, April 16-20, 2005 (Abstract #4563), at 96th Annual Meeting of AACR.

15. Languages spoken: Georgian, English, Russian